REMARKS

Applicants thank the Examiner for acceptance of the Request for Continued Examination, withdrawal of the finality of the previous Office Action, and entry of previous Response filed April 1, 2004. Claims 2, 4-5, 8-14, 17-20 and 24-69 are pending in the present application.

Claims 24-26, 46, 47 and 61-63 are amended. The amendments to the claims are supported throughout the specification, for example at page 13, line 14, 29-31.

Entry of this Amendment and reconsideration of the rejection of the claims is respectfully requested.

35 U.S.C. § 112 ¶1

Claims 24-36, 38-41, 43-45, and 61-69 are rejected under §112, ¶1 for failing to comply with the written description requirement. The Examiner contends that claimed concentrations, from equal or less than 1pg/ml (which encompasses zero) to 1000 pg/ml, and/or concentration ranges of target molecules, 0.03 – 5000 pg/ml, are not taught or suggested by the specification. The Examiner notes that the specification teaches concentration ranges of 0.005-5000 pg/ml and preferably 1-1000 pg/ml at page 23, lines 31-34. The rejection under §112¶1 is respectfully traversed.

The written description requirement is satisfied when Applicants' specification conveys with reasonable clarity to those skilled in the art, that as of the filing date sought, he or she was in possession of the invention. Vas-Cath Inc. v. Mahurkar, 19 USPQ2d 1111, 1116 (Fed. Cir. 1991). It is not necessary that the application describe the claim limitations exactly, but only so clearly that persons of ordinary skill in the art would recognize from the disclosure that applicant's invention included those limitations. In re Smythe, 480 F.2d 1376, 178 USPQ 179 (CCPA 1973). For example, the disclosure of a temperature range of 60°C to 200 °C and specific disclosure of heating to 80 °C was sufficient description for a claim containing a range of 80 °C to 200 °C. In re Blaser, 194 USPQ 122 (CCPA 1977).

The claimed methods are directed to detecting or quantitating a target molecule. The specification teaches that target molecules present in a sample at concentrations between 0.005-

5000 pg/ml can be detected by the claimed methods. Specific examples of concentrations detected by the claimed methods are also provided in the specification. For example, 0.41 pg/ml to 100 pg/ml at page 35, line 17; 0.031 pg/ml to 1 pg/ml, and 3.1 pg/ml to 1000 pg/ml at page 35, lines 30-31; Figure 5 shows detection in the range of 0.031 to 1000 pg/mL; See also, page 13 lines 29-31, which notes detection at target molecule concentrations less than 1.0×10^{-12} grams/mL (1 pg/mL) and generally from about 1.0×10^{-15} grams/mL to about 1.0×10^{-8} grams/mL (0.001pg/mL to 10 ng).

The range of detectable target concentrations is a measure of the sensitivity of the detection method. The description of the maximum range of detectable concentrations, as well as the demonstrated examples, provides sufficient guidance for one to appreciate the claimed ranges of detection. For example, where the range of detectable target concentrations over 0.005-5000 pg/ml is supported by the description, the claimed detection method is also recognized as working at 0.4-5000pg/ml, 0.03-5000pg/ml and 100-5000pg/ml.

This position is consistent with the Board decision in McLaughlin v. Roberts. Roberts' claims required 10-25% propellant. The disclosure specified that the propellant may be present from 10-79% of the product, preferably 40-79%, and more preferably 40-60%. The 25% upper limit of the claimed range was not specifically disclosed. The Board held that one of ordinary skill in the art, given appellant's disclosure, would consider the use of the 10-25% range as a part of the invention. The written description requirement was satisfied. Likewise, the disclosure was considered enabling even though the 10-25% was not disclosed as the preferred part of the range and there were no examples of percentages within the lower range because one skilled in the art would be able to practice the invention using compositions having a propellant content within that range. McLaughlin v. Roberts, 197 USPQ 831, 835 (Bd. Pat. Int. 1978)

An alternative way of describing the detectable target concentrations is by "less than 1000pg/ml, etc .. These ranges are also encompassed by the broader range described in the specification, and detection of target molecule concentrations less than 1.0×10^{-12} grams/mL (1 pg/mL) is specifically stated on page 13. The Examiner is concerned the specification does not teach target concentrations encompassing zero. The methods can be used on a sample having a zero concentration of the target molecule, in which case no target molecule is detected. It is

generally assumed with the use of any detection method, that a "negative" control containing no detectable sample is used.

Reconsideration and withdrawal of the rejection for lack of written description is requested.

35 U.S.C. § 112 ¶2

Claims 24-26, and 61-63 are rejected under §112, ¶1 as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. The Examiner finds use of the phrase "less than about 1000 (100 and/or 1) pg/ml" vague and indefinite. While not aquiescing to the rejection and solely to expedite prosecution, the claims are amended to remove the term "about" to alleviate the rejection. Applicants respectfully request withdrawal of the rejection.

35 U.S.C. § 103(a)

Claims 2, 4-5, 8-14, 17-20 and 24-46 are rejected under 35 U.S.C. § 103(a) as being unpatentable over Hendrickson in view of Gibson and Gold. The Examiner contends that Hendrickson teaches a method comprising forming a capture molecule - target complex, adding a nucleic acid moiety containing a detector molecule specific for the target, washing the complex to remove test materials (e.g. nucleases) and amplifying and quantifying nucleic acid by PCR. The Examiner contends Gibson discloses the use of real-time PCR using sequence specific non-primer probes. The Examiner also contends that Gold teaches that aptamers have many advantages over antibodies and can readily be employed in assays in place of antibodies. Applicants respectfully traverse.

Applicants claims are directed to a method for detecting a target molecule in a sample that may contain a target molecule and a nuclease comprising:

- exposing the sample to a capture antibody or target molecule binding fragment thereof

  - whereby a capture antibody, or fragment thereof, forms a complex with the target molecule;
- adding to the complex from step (a) an RNA or DNA aptamer detector molecule which binds to the target to form a ternary complex;
- washing the complex from the first or second step or both to remove nucleases;
- amplifying the aptamer by PCR and quantitating or detecting the PCR amplified DNA using a detectable non-primer probe and real-time PCR;
  - wherein quantitating or detecting PCR amplified DNA quantitates or detects the target molecule.

In another embodiment, the method provides for detection of target molecules when present at a concentration of about 0.005 to about 5000 pg/mL.

In order to establish a prima facie case of obviousness, three basic criteria must be met, namely: 1) a suggestion or motivation to modify the references or combine the reference teachings; 2) the references when combined must teach or suggest all of the claim limitations; and 3) the references when combined must provide a reasonable expectation of success. Applicants submit that all of these requirements have not been met.

The Examiner reiterated in the office action, that it is the combination, not the individual references that teach the instant invention. Further stating, "In response to applicant's arguments against the references individually, one cannot show nonobviousness by attacking references individually where the rejections are based on combination of references." Applicant's agree the proper standard for a prima facie case of obviousness requires teaching of the claimed invention, by properly combined references, as described above. Applicant's arguments are not individual attacks on each reference. The arguments are directed to the portion of the combination taught by the indicated reference(s). Applicant asserts this format for response should be acceptable as the Examiner builds the rejection in a similar manner, relying on and describing specific teachings of each reference.

1. <u>The Examiner has not established that there is a motivation to combine the cited references or modify the Hendrickson et al. reference with the Gibson et al. and Gold et al. references.</u>

Motivation to combine the cited references has not been established and evidence that teaches away from the claimed methods has not been properly taken into account. In fact, "[A] rejection cannot be predicated on the mere identification . . . of individual components of the claimed invention. Rather, particular findings must be made as to the reason the skilled artisan, with no known knowledge of the claimed invention, would have selected these components for combination in the manner claimed." <u>Ecolochem Inc. v. Southern Calif. Edison Co.</u>, 227 F3d 1361, 1375 (Fed. Cir. 2000). "Obvious to try" is not the standard. <u>Ecolochem</u> at 1374.

The cited references do not provide motivation, either implicitly or explicitly, to combine these references.

> Obviousness can only be established by combining or modifying the teachings of the prior art to produce the claimed invention where there is some teaching, suggestion, or motivation to do so found either explicitly or implicitly in the references themselves or in the knowledge generally available to one of ordinary skill in the art. "The test for an implicit showing is what the combined teachings, knowledge of one of ordinary skill in the art, and the nature of the problem to be solved as a whole would have suggested to those of ordinary skill in the art." In re Kotzab, 217 F.3d 1365, 1370, 55 USPQ2d 1313, 1317 (Fed. Cir. 2000). See also In re Lee, 277 F.3d 1338, 1342-44, 61 USPQ2d 1430, 1433-34 (Fed. Cir. 2002) (discussing the importance of relying on objective evidence and making specific factual findings with respect to the motivation to combine references); In re Fine, 837 F.2d 1071, 5 USPQ2d 1596 (Fed. Cir. 1988); In re Jones, 958 F.2d 347, 21 USPQ2d 1941 (Fed. Cir. 1992).

MPEP, 8th Ed., Rev. 2, May 2004, §2143.01.

The Hendrickson et al. reference is directed to an immunoglobulin sandwich assay with covalently bound DNA for detection by PCR of multiple analytes. The reference itself does not teach or suggest that the described antibodies could be substituted with aptamers. There is also no teaching or suggestion that PCR can or should be substituted with real-time PCR.

The reference does discuss alternative methods of detection, but those alternative methods of detection were using direct detection of ligands, for example by the use of enzyme

labels. The reference also discusses that previous methods were undesirable because complexes between DNA and antibodies were assembled *in situ*, and, therefore, had variable stoichiometry (see col. 2, page 522). Thus, the reference suggests that the DNA directly coupled to an antibody was essential and teaches away from forming DNA complexes in situ. The reference further indicates that extra steps are required for the addition of biotinylated reagents and numerous wash steps are required to remove non-specifically bound reagents. The authors conclude previous assays have been too complex and require too much hands on time.

Hendrickson also states that antibody reagents labeled with fluorescent labels were not practical, because of overlapping signals from different labels and difficulties in discriminating signal intensities at various analytic concentrations. Quantitation and sensitivity are affected (page 527, 2nd column, 3rd paragraph), thereby indicating that not all other detection methods are suitable or can easily be substituted into immuno PCR. These statements teach away from substitution of real time PCR using fluorescently labeled reagents as a means of detection. In addition, Hendrickson et al. discusses the simplicity of their method and minimizing the number of handling steps (p. 528 bottom of column 1 and top of column 2).

Biological samples, such as blood, contain a mixture of components and may include nucleic acid degradative activity (i.e. nucleases). The methods of Hendrickson are preformed using purified protein targets, purified antibodies and other purified reactants. Because of the nuclease-free *in vitro* environment, Hendrickson does not realize or discuss the problem of nuclease contamination. How nucleases might affect adaptation of Hendrickson's method to utilize aptamers and real-time PCR is not considered. Although Hendrickson detects a protein that is natively found in blood, the protein is not detected from a sample that is blood. The protein is purified and presented in Hendrickson's method as one of three potential binders. The detection from complex samples (e.g., blood) is not supported or suggested.

Gibson et al. reference also does not provide any motivation to be combined with Hendrickson and/or Gold. Gibson describes use of real-time PCR to detect mRNA samples for use in detecting expression in gene therapy. Gibson et al. does not teach or suggest this assay should be substituted for other PCR assays in other methods. Gibson describes an assay that is complicated and requires more steps than Hendrickson, which values simplicity. Applicants

submit that one of skill in the art would not be motivated to substitute this method of detection in the methods of Hendrickson due to the increase in complexity.

Moreover, the Gibson teaches using a hybridization probe that is fluorescently labeled. Under certain circumstances, a lack of sensitivity and dynamic range can result due to overlapping fluorescent spectra or high background, as further discussed in Hendrickson. In addition, the problem of several unknown variables such as forming aptamer target complexes in situ and high background fluorescence as recognized by Hendrickson et al. would teach away from using real time PCR. In addition, Gibson employs internal control DNA that may not be readily incorporated into immuno PCR reactions, especially those involving biological samples. Finally, Gibson is silent regarding the problem of nucleases (nuclease degradation of nucleic acids) and other contaminants in biological samples. The problems associated with the presence of nucleases or the affect of the presence of nucleases on the sensitivity and/or dynamic range of the assay is not recognized, nor addressed in Gibson.

The Gold et al. reference also does not provide motivation to be combined with Hendrickson and/or Gibson. Gold describes the selection and identification of aptamers and further suggests that aptamers can be employed in the same assays as antibodies under conditions where nucleic acids are resistant to degradation. (Col. 27, lines 50-60). How aptamers are to be substituted for antibodies is not further described. Also, lacking is description of conditions where nucleic acids are degraded or how to avoid those conditions. In addition, Gold does not suggest substituting detection methods used in immuno PCR with those of real-time PCR. There is no showing in Gold that either the substitution of aptamers for antibodies, or immuno PCR for real-time PCR can be successfully made. There is no teaching that the sensitivity and/or dynamic range of the claimed assay would be achieved, especially in biological samples. Thus, Applicants submit one of skill in the art would not be motivated by Gold to employ aptamers in the PCR assay of Hendrickson in samples containing nucleases. Moreover, Gold is silent on the use of real-time PCR as a substitute for PCR in an immuno assay.

As discussed previously, the Williams et al. reference teaches away from the use of aptamers, by indicating that use of aptamers is limited by their sensitivity to nucleases in biological samples.

The Examiner contends that Gold teaches washing of complexes to produce amplifiable nucleic acids. However, the section of the patent cited by the examiner, is directed to releasing the selected aptamers from a bound complex with protein that have been bound to nitrocellulose filters. There is no teaching or suggestion of the need for a wash step to remove nucleases or other interfering molecules. Instead, the step taught by Gold is directed to recovering the aptamer for amplification and identification of the selected aptamer that is specific for that particular protein.

Furthermore, the above teaching of Gold teaches away from substituting into the method of Hendrickson. Hendrickson does not disassociate the detector molecule from the target molecule for detection. Gold suggests that the aptamer must be released from binding to the target protein before detection by PCR can be performed. Hence the combination of Hendrickson with Gold does not teach the aptamer detection molecule of the present invention. The claimed method does not require release and purification of the aptamer for detection as required by the methods of Gold. Finally, neither Hendrickson, nor Gold suggest substituting real-time PCR techniques for the standard PCR techniques taught.

In summary, Applicants submit that there is no motivation to combine the cited references. The cited references do not provide the motivation to substitute both the antibodies and PCR of Hendrickson with the aptamers of Gold et al. and real-time PCR of Gibson et al. In fact, Williams et al. teach away from substituting aptamers in situations where degradation might be present, such as due to nucleases in biological samples. Moreover, Hendrickson et al. also discusses the disadvantages of forming nucleic acid antibody complexes that are assembled in situ, and further teaches away from complex systems. In addition, Applicants submit that one of skill in the art would not be motivated to substitute the real-time PCR of Gibson et al. for that of Hendrickson et al., because it is more complicated, uses fluorescently labeled probes that can affect sensitivity and uses internal competitor DNA that may also affect sensitivity when using biological samples.

The Examiner repeatedly states that the methods taught by Gold et al. and Gibson et al. were known in the art and therefore it would be obvious to one of skill in the art to substitute the methods of Gold and Gibson into the method of Hendrickson. Obvious to try is not a proper

rationale for combining references. The references themselves do not provide suggestion, either implicitly or explicitly, to combine the methods taught by Gold et al., Gibson et al. and Hendrickson. The Examiner has also not taken into account information in the references that would lead one of skill in the art from combining these references. Thus, Applicants respectfully request withdrawal of the rejection on this basis.

2. <u>The references when combined do not teach all of the elements of the claimed invention.</u>

Applicants submit that even when all of the references are combined, they do not disclose all of the elements of the claimed invention.

Applicants claims are directed to a method for detecting a target molecule in a sample that may contain the target molecule and a nuclease comprising:

- exposing the sample to a capture antibody
  - whereby a capture antibody forms a complex with the target molecule;
- adding to the complex from step (a) an RNA or DNA aptamer detector molecule which binds to the target to form a ternary complex;
- washing either complex or both to remove nucleases;
- amplifying the aptamer by PCR and quantitating or detecting the PCR amplified DNA using a detectable non-primer probe and real-time PCR;
  - wherein quantitating or detecting PCR amplified DNA quantitates or detects the target molecule.

Applicants also claim a method that can detect the presence of a target in a sample at a concentration of 0.005 to 5000 pg/mL.

The cited combination of references does not disclose a method directed to detecting a target in a sample:

- that may contain the target and a nuclease, and
- including an RNA or DNA aptamer detector molecule, and
- quantitating the PCR amplified DNA by real-time PCR.

Hendrickson does not teach the use of RNA or DNA aptamer detector molecules. Hendrickson does not contemplate, teach or suggest the use of aptamers in order to capture and

detect the target molecule. Moreover, Hendrickson et al. indicates that formation of antibody DNA complexes in situ is not desirable. Additional considerations in the use of aptamers, such as the presence of nucleases and the need to remove nucleases is not addressed by Hendrickson. Moreover, Hendrickson suggests that washing steps can and should be eliminated in order to simplify the assay described therein further. See page 528, second column, first full paragraph. Hendrickson also does not teach the quantitation of DNA PCR products by RT-PCR utilizing a detectable non-primer probe which binds to the DNA.

The deficiencies of the Hendrickson reference are not remedied by reference to Gibson and Gold. Gibson is also silent on the use and/or amplification of aptamers and does not discuss any concerns relating to nucleases in the sample. Gold discusses the use of aptamers in assays for detecting target molecules and discloses that the conditions under which the aptamer can be used, the nucleic acid is substantially resistant to degradation. (Col. 27, lines 50-55).

Gold does not teach or suggest that a washing step to remove nucleases would be sufficient and should be employed to remove nucleases to protect the aptamer from degradation. Furthermore, the above teaching of Gold suggests that the aptamer must be released from binding to the target protein before detection by PCR can be performed. In the detection of the present invention, the aptamer is not required to be released from the target for detection. Hence the combination of Hendrickson with Gold does not teach the aptamer detection molecule of the present invention with direct detection of the aptamer bound to the target molecule, and do not teach substituting Real-time PCR techniques for the standard PCR.

Thus, Applicants submit that even when all of the references are combined, they do not teach all of the elements of the claimed invention, in particular, washing the complexes to remove nucleases, and detecting aptamers using real time PCR.

The references, in combination, do not teach a method for detecting a target molecule in a sample at target molecule concentrations of 0.005 to 5000 pg/mL, or any additional sample target molecule concentrations in claims 61-69. Neither Gibson, nor Gold, describe a relevant assay or sensitivity thereof. Hendrickson teaches that immuno PCR is a sensitive assay and can detect target molecules at 1 fg using antibodies for both capture and detection. However, the accuracy of methods utilized by Hendrickson is questionable.

> In immuno-PCR, antigen concentrations are generally determined by post PCR analysis of the reporter amplicon by either gel electrophoresis or PCR-ELISA. Quantitation of the DNA label by analyzing the endpoint PCR product is prone to errors since the rate of product formation decreases after several cycles of logarithmic growth (Ferre, 1992; Raeymakers et al., 1995) and the post PCR sample handling may lead to laboratory contamination. In addition, these methods require multiple steps and washes, during which the antibody:antigen complex may dissociate (Tijssen, P., ibid.).

Background at page 5, lines 15-21. This is especially true when the sample is a biological sample and may contain nucleases or other interfering substances. However, there is no teaching or suggestion in the combination of cited references that an antibody used to detect the target molecule (Hendrickson et al.) can be replaced with a aptamer and that the PCR can be replaced by detection of aptamer with a non-primer probe and real-time PCR and maintain the ability to detect target molecules at picogram levels. In fact, Hendrickson suggests that formation of DNA antibody complexes in situ leads to variable stoichiometry in the assembly of components and in the attachment of the DNA label.

Gibson shows that real-time PCR can be sensitive to detecting low copy numbers of mRNA. But, Gibson does not teach or suggest that the same sensitivity can be obtained when the nucleic acid to be detected is an aptamer and the aptamer is bound to an antibody target molecule complex in a sample that may contain nucleases or other interfering substances.

Similarly, Gold does not teach or suggest that aptamers could be substituted in an assay for detection of a target molecule and achieve the same level of sensitivity for detection of a target molecule in a sample that may contain nucleases or other interfering substances. There are no working examples in Gold showing that an aptamer can be substituted in an assay for detecting a target molecule and achieve the same level of sensitivity as the claimed methods.

Thus, Applicants submit that the cited references either alone, or in combination, do not disclose all of the elements of the claimed invention. In the least, the cited references, when combined, do not teach or suggest a method for detecting a target molecule in a sample utilizing real-time PCR detection of a bound aptamer detection molecule; that the sample may contain nucleases, including a step to wash the complexes to remove nucleases; and do not teach or

suggest a method that has the dynamic range of detection in biological samples as claimed by Applicants.

3. The references do not provide a reasonable expectation of success.

Applicants claims are directed to an assay for detecting a target molecule in a sample that may include nucleases using a detection method using an aptamer and real-time PCR. Applicants have also claimed an assay that detects a target molecule in a biological sample at a range of concentration of 0.005 pg/mL to 5000 pg/mL.

Applicants submit that none of the cited references teach or suggest that the antibody of Hendrickson can be substituted with an aptamer, especially in samples that may contain nucleases to detect and sensitively detect or quantitate the target molecule. Both Hendrickson and Gibson are silent on whether aptamers could be utilized in immuno PCR assay. Gold teaches that aptamers can be utilized in assays in place of antibodies, but under conditions where the nucleic acids can avoid degradation. Williams teaches away from the use of aptamers when samples contain nucleases. Moreover, biological samples could contain other materials that could inhibit or degrade aptamers. Thus, Applicants submit that one of skill in the art would not have a reasonable expectation of success in using aptamers as substitutes for antibodies in an assay for detecting target molecule in a biological sample or in a sample that may contain nucleases.

In addition, the combination of cited references do not teach or suggest that both the antibodies and the PCR assay of Hendrickson could be substituted with aptamers and real time PCR and achieve the sensitivity and dynamic range of detection as claimed by Applicants. Hendrickson teaches that addition of more steps, reagents and washing can decrease sensitivity and that not all detection methods provide the same sensitivity, especially those involving fluorescent labeled reagents (page 527, col. 2, 3rd paragraph; p. 528, col. 2, top of page and first paragraph). Hendrickson also teaches that formation of DNA antibody complexes in situ can lead to variable stoichiometry and require multiple wash steps. (See page 522, col. 2, 2nd paragraph.) Gibson method includes multiple steps and discusses the sensitivity of the assay may also be limited by overlapping spectra of the reporter dyes (p. 996, col. 1, 2nd paragraph).

Gibson also employs internal competitor DNA that may interfere with the sensitivity of detection in complex biological samples. Finally, substituting aptamers for DNA labeled antibodies in biological samples that may contain nucleases, can limit the sensitivity of the assay. Thus, when all of these references are considered as a whole, one would not have a reasonable expectation of success that substitution of both aptamers (Gold) and real-time PCR (Gibson) in the method Hendrickson would provide an assay for detecting a target molecule with a high degree of sensitivity, especially in a biological sample.

Regarding claim 50, the Examiner indicates that "Hendrickson et al. teach the method wherein the capture molecule is bound to a solid support (page 1372, Fig. 1) but they do not teach the method wherein the capture molecule is in solution during step (a) or (b)." The Examiner further asserts as the basis for a finding of obviousness that " binding of capture molecules in solution is routinely practiced in the art. " Applicants respectfully traverse. It is the Examiner's burden to present evidence to meet the prima facie standard for obviousness. Applicant's assert that the Examiner has not met this burden by failing to provide evidence of a capture molecule that is not bound to a solid support, for use in combination with the references cited above.

Applicants also submit that claimed methods achieve unexpected results. Applicants submit that the combination of an aptamer detector molecule with real time PCR, provides an assay that is both very sensitive and has a broad dynamic range, especially with biological samples that may have many interfering substances. Applicants hereby submit Barletta et al, reference. This reference shows that an assay employing a DNA construct and using real time PCR can be used to sensitively detect HIV p24 antigen in biological samples. Thus, for at least theses reasons, Applicants submit that the claimed methods are not obvious.

Based on the foregoing, Applicants respectfully request withdrawal of the 35 U.S.C. § 103 rejection.

35 U.S.C. § 103(a)

The Examiner rejected claims 2, 4, 5, 8-14, 17-20 and 46 under 35 U.S.C. § 103(a) as being unpatentable over Cubicciotti in view of Gibson. The Examiner contends that Cubicciotti teaches a method for detecting a target using a capture molecule, adding to the complex a nucleic acid containing a detector molecule, washing the complex and amplifying nucleic acid moiety (aptamer) by PCR and quantitating or detecting PCR amplified material. The Examiner further contends Gibson teaches using real-time PCR with a detectable non-primer probe. Applicants respectfully traverse.

Applicants claims are directed to a method for detecting a target molecule in a sample that may contain the target molecule and a nuclease comprising exposing the sample to a capture antibody or target molecule binding fragment thereof whereby a capture antibody, or fragment thereof, forms a complex with the target molecule; adding to the complex from step (a) an RNA or DNA aptamer detector molecule which binds to the target to form a ternary complex; washing the complexes to remove nucleases; amplifying the aptamer by PCR and quantitating or detecting the PCR amplified DNA using a detectable non-primer probe and real-time PCR; wherein quantitating or detecting PCR amplified DNA quantitates or detects the target molecule. In another embodiment, the method provides for detection of target molecules when present at a concentration of about 0.005 to about 5000 pg/mL.

In order to establish a prima facie case of obviousness, three basic criteria must be met, namely: 1) the references when combined must teach or suggest all of the claim limitations; 2) a suggestion or motivation to modify the references or combine the reference teachings; and 3) a reasonable expectation of success. Applicants submit that all of these requirements have not been met, in the least because, the cited references when combined do not disclose all of the elements of the claimed invention.

Applicants submit that the Cubiciotti reference does not disclose an assay for detecting a target in a sample, that may contain nucleases, employing a capture antibody and an aptamer detector molecule, washing to remove nucleases, quantitating or detecting the aptamer using real-time PCR quantitating or detecting the target molecule.

The section of the patent cited by the Examiner is in Example 18 entitled "Selection of a Synthetic Defined Sequence Segment for the Ability to Stabilize a Peptide Drug". (Col. 227, lines 63-65). The patent further indicates the example describes selecting a defined sequence segment that binds a therapeutic peptide and attenuates peptide degradation (col. 228, lines 20-30, below).

> For example, a defined sequence segment can be selected to bind a therapeutic peptide and attenuate peptide degradation under physiological conditions, thereby increasing the in vivo half-life and therapeutic efficacy of the nucleotide-bound peptide.

The method described at column 229, lines 5-52 is a method for selecting an aptamer that decreases the degradation of chimeric F(ab) fragment anti-7E3 (col. 229, lines 15-41 and 50-54, reproduced below). The F(ab) fragment anti-7E3 is the therapeutic protein used to inhibit platelet aggregation. In contrast to the assertion of the examiner, the therapeutic protein is not peptide 7E3 but rather is the antibody fragment itself. The method of this example involves selecting aptamers that bind to the protein and prevent degradation of the protein. The selected aptamers were isolated by affinity chromatography and were amplified and sequenced(col. 229, lines 34-60).

> A library of fluorescein-labeled RNA molecules comprising a 35-nucleotide randomized sequence flanked by PCR primer sequences is produced by transcription of a corresponding cDNA array. The receptor-specific chimeric Fab fragment anti-7E3 (CENTORX; Centocor, Malvern Pa.) is incubated with the library for two hours at room temperature, and the mixture is transferred to a screw-capped flask comprising cultured fibroblasts in human serum protein-supplemented growth medium. After 24-hours in a 37.degree. C., controlled CO.sub.2 incubator, the medium is transferred to microfuge tubes and centrifuged at 8,000.times.g for one minute to remove cells, aggregates and debris. The supernatant is transferred to fresh microfuge tubes and **fluorescein-labeled anti-7E3/nucleotide complexes** are separated from the remainder of the mixture by agarose-RGDS affinity chromatography (and/or ion-exchange chromatography) with fluorescence monitored in black FluoroNunc.TM. plates (Nunc, Inc. Naperville, Ill.) using a FLUOSTAR microplate fluorimeter (SLT Labinstruments, Research Triangle Park, N.C.). **Only aptamers that specifically bind the anti-7E3 Fab fragment and protect from enzymatic degradation in enzyme-supplemented medium are isolated by the selection procedure.** Unbound fluorescein-labeled RNA sequences, fluorescent nucleotide fragments, and anti-7E3-binding nucleotides that fail to protect against anti-7E3 epitope

modification in enzyme-supplemented culture are retained by the chromatography medium. (col. 229, lines 15-41)

Selected aptamers are amplified by PCR and sequenced. (col. 229, line 50.)

This process does not disclose a capture antibody for binding to a target molecule.

This process does not disclose a detectable aptamer that binds target molecule in a target molecule:capture antibody complex, washing to remove nucleases.

This process does not disclose amplifying the aptamer, quantitating or detecting the PCR amplified DNA using a detectable non-primer probe.

This process does not disclose quantitating or detecting the PCR amplified DNA to quantitate or detect the target molecule.

Moreover, since this method is not a method to detect a target molecule and because there are no actual examples, there is no discussion of detecting or quantitating the target molecule in a range of .005 pg/mL to 5000 pg/mL.

The deficiencies of Cubiciotti are not remedied by reference to Gibson. Gibson does not disclose using a capture antibody or an aptamer detector molecule that binds to the target molecule or washing the complexes to remove nucleases. Gibson discusses detection of a mRNA molecule, but does not teach or suggest that the level of detection could be maintained in other assays. Thus, Applicants submit the cited references when combined do not teach or suggest all of the elements of the claimed invention. Applicants, therefore, request the withdrawal of the rejection of the claims on this basis.

The Examiner also rejected claims 24-45 as being unpatentable over Cubiciotti in view Gibson, and further in view of Hendrickson. The Examiner contends that Cubiciotti teaches the method of claim 46, but is silent regarding the concentration of the target molecule. The Examiner contends that it would be obvious to apply the well known detection sensitivity of Hendrickson to detect molecules at a concentration equal to or less than 1 pg/mL. Applicants respectfully traverse.

Applicants submit Cubiciotti does not teach the method of claim 46. As discussed previously, the cited portions of Cubiciotti are directed to a method of selecting an aptamer that

binds to a protein and prevents degradation of this protein. The protein is a F(ab) fragment of anti-7E3. This protein is the protein to be protected and does not serve as the capture antibody. Moreover, the aptamer is amplified by PCR, not to quantitate or detect the protein, but to obtain aptamer for use in conjunction with the antibody to prolong half-life of antibody fragment *in vivo*.

The deficiencies of the Cubiciotti et al. reference are not remedied by reference to Hendrickson or Gibson. There is no teaching or suggestion in either reference that the antibodies and PCR of Hendrickson could be substituted with aptamers and real-time PCR, and achieve the same level of sensitivity, especially in samples that may contain nucleases.

Thus, Applicants respectfully request reconsideration and withdrawal of the rejection on this basis.

Request for an Interview

Applicants respectfully request an interview with the Examiner and her supervisor. Applicants request that the Examiner contact Applicants' representative upon receipt of these papers to schedule an interview.

SUMMARY

Applicants submit that the claims are in condition for allowance and notification to that effect is earnestly solicited. An interview with the Examiner is requested.

Respectfully submitted,

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